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Neuroinflammatory Markers Associate with Cognitive Decline after Major Surgery: Findings of an Explorative Study

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Objective: Long-term cognitive decline is an adverse outcome after major surgery associated with increased risk for mortality and morbidity. We studied the cerebrospinal fluid (CSF) and serum biochemical inflammatory response to a standardized orthopedic surgical procedure and the possible association with long-term changes in cognitive function. We hypothesized that the CSF inflammatory response pattern after surgery would differ in patients having long-term cognitive decline defined as a composite cognitive *z* score of ≥1.0 compared to patients without long-term cognitive decline at 3 months postsurgery.
Methods: Serum and CSF biomarkers of inflammation and blood–brain barrier (BBB) integrity were measured preoperatively and up to 48 hours postoperatively, and cognitive function was assessed preoperatively and at 2 to 5 days and 3 months postoperatively.
Results: Surgery was associated with a pronounced increase in inflammatory biomarkers in both CSF and blood throughout the 48-hour study period. A principal component (PC) analysis was performed on 52 inflammatory biomarkers. The 2 first PC (PC1 and PC2) construct outcome variables on CSF biomarkers were significantly associated with long-term cognitive decline at 3 months, but none of the PC construct serum variables showed a significant association with long-term cognitive decline at 3 months. Patients both with and patients without long-term cognitive decline showed early transient increases of the astroglial biomarkers S-100B and glial fibrillary acidic protein in CSF, and in BBB permeability (CSF/serum albumin ratio).
Interpretation: Surgery rapidly triggers a temporal neuroinflammatory response closely associated with long-term cognitive outcome postsurgery. The findings of this explorative study require validation in a larger surgical patient cohort.

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Long-term decline in cognitive capacity is a feared adverse outcome after surgery, affecting 10 to 20% of elderly surgical patients and associated with increased risk for mortality and morbidity.[1–4] Recent advances in experimental and translational human models point toward a critical role for the innate immune system behind this long-term impact on higher brain functions.[5,6]

Surgical trauma triggers the innate immune system to launch a systemic inflammatory response orchestrated by humoral and cellular inflammatory mediators that ultimately promote healing and restoration of homeostasis. Although initiated locally by damage-associated molecular patterns (DAMPs), the immune response to trauma is rapidly transmitted systemically to remote organs by humoral mediators and by activated peripheral immune cells. Despite the classical view of the brain as an immune-privileged organ, there is a growing body of evidence that the central nervous system (CNS) is impacted by surgical trauma by inflammatory mediators rapidly reaching the brain via cellular and humoral signaling, facilitated by a transient increase in blood–brain barrier (BBB) permeability.[5] Activation of the CNS immune system by surgery and tissue damage is a hallmark of sickness syndrome being proposed to further develop to cognitive decline postsurgery, especially in the elderly.[1,3,4]

A series of studies in humans suggests that surgery is followed by a rapid (within 4–8 hours) elevation in cerebrospinal fluid (CSF) inflammatory, astroglial, neuronal degenerative, and injury biomarkers[7–11] with simultaneous signs of BBB damage[8,12] along with transient increase in systemic concentrations of neurodegenerative and brain injury biomarkers (tau and neurofilament light).[13] Recent positron-emission tomography (PET) findings among surgical patients have furthermore uncovered marked changes in brain immune activity with an association between long-term upregulation of human brain immune activity postsurgery and a simultaneous reduction in higher cognitive brain functions.[14]

In this explorative study, we mapped the immediate temporal CSF and serum biochemical inflammatory response trajectory to a standardized surgical procedure and explored the potential association between a temporal molecular pattern and long-term changes in cognitive function. We hypothesized that the individual CSF inflammatory response trajectory after surgery would differ in patients with, versus those without, long-term postoperative cognitive decline.

Patients and Methods

Participants and Study Design

This study complies with the 2013 Declaration of Helsinki. The Regional Ethics Committee in Stockholm, Sweden, approved the study protocol (Dnr 2013/2297-31/4 and 2014/834-32), and the study was registered at www.clinicaltrials.gov (identifier NCT02759965).

Between September 2014 and March 2016, we included 34 patients scheduled for elective total hip or knee replacement surgery in this prospective observational clinical trial at Karolinska University Hospital, Stockholm (n = 14), and Sahlgrenska University Hospital, Mölndal (n = 20), after obtaining signed informed and written consent. Exclusion criteria included preexisting neurological, psychiatric, or clinically evident neurovascular disease, recent or ongoing treatment with anti-inflammatory drugs, severe organ failure (eg, cardiac, renal, or hepatic), coagulopathy, alcohol or drug abuse, poorly controlled diabetes mellitus, or autoimmune disease. Patients with preoperative cognitive impairment, corresponding to a Mini-Mental State Examination score of <24, were excluded. Prior to anesthesia induction, a polyamide lumbar intrathecal catheter (Perifix epidural catheter; Braun, Melsungen, Hessen, Germany) was inserted using an 18-gauge Tuohy needle. Spinal anesthesia was induced with 10 to 15mg bupivacaine (Marcain Spinal; Astra-Zeneca AB, Södertälje, Sweden) and 5 to 10μg sufentanil (Sufenta; Janssen-Cilag AB, Solna, Sweden) administered via the intrathecal catheter and supplemented with light intravenous (IV) propofol sedation (Propolipid; Fresenius Kabi AB, Uppsala, Sweden) in IV bolus doses of 10 to 30mg or an infusion at a rate of 0.5 to 2mg/kg/h. None of the patients received oral or IV anti-inflammatory and psychoactive drugs intraoperatively. Six patients (22%) received subcutaneous ketorolac 15 to 30mg intraoperatively as part of the postoperative pain regimen.

The intrathecal catheter was left in place for 48 hours. Preoperatively and at 4, 8, 24, 32, and 48 hours after skin incision, serial CSF (5ml) and blood samples (20 ml) were collected and subsequently centrifuged, aliquoted, and stored at −80°C for offline analysis.

CSF and Serum Biomarkers of Systemic and Neuroinflammation

CSF and serum samples were analyzed using a high-throughput, multiplex immunoassay analysis (Proseek Multiplex, Proximity Extension Assay technology, Inflammation 1 panel; Olink, Uppsala, Sweden). This panel was compiled to detect established and exploratory biomarkers of inflammation. Oligonucleotide-labelled antibody probe pairs were allowed to bind to their respective target protein present in the sample. A polymerase chain reaction (PCR) reporter sequence was formed by a proximity-dependent DNA polymerization event, amplified, and subsequently detected and quantified using real-time PCR. Analyzed proteins are described in Supplementary Table 1. Values of the various inflammatory variables are

presented as an arbitrary unit in log2 scale and can thus be used only for relative changes of the same protein.

CSF and Serum Biomarkers of Astroglial Injury and BBB Dysfunction

Measurements of the astroglial injury markers S-100B (CSF, serum) and glial fibrillary acidic protein (GFAP; CSF) were performed by an electrochemiluminescence immunoassay and enzyme-linked immunosorbent assay,[15] respectively, and albumin levels in CSF and serum were measured by immunonephelometry.

Neurocognitive Testing

Neurocognitive capacity was assessed using the International Study of Postoperative Cognitive Dysfunction test battery[4] 1 to 2 weeks prior to surgery, at discharge from the hospital (2–5 days after surgery), and at 3 months after surgery. In brief, this test battery measured cognitive performance using 4 different tests, providing 7 variables for analysis.[4] Changes in cognitive performance were calculated as previously described[4] for each of 7 test variables and corrected for practice effects and variability using data from an age-matched control group who underwent testing using the same battery and with the same intervals. To quantify the change from preoperative to postoperative tests, z scores were calculated for each variable. We defined long-term cognitive decline as a composite z score of ≥1.0 at 3 months postsurgery (poor neurocognitive outcome group), and those without had a composite z score of <1.0 (good neurocognitive outcome group).

Sleep, Pain, and Delirium Assessment

A visual analog scale (VAS) expressed as a 10-point scale was used to assess quality of sleep and the severity of postoperative pain at regular intervals after surgery. Delirium was assessed 24 and 48 hours postoperatively using the confusion assessment method for the intensive care unit (CAM-ICU).[16]

Statistical Analysis

The sample size calculation was based on the hypothesis that there is a significant association between the postoperative cytokine concentration in CSF and a change in cognitive function, expressed as the combined z score at the first postoperative test. We assumed that the correlation coefficient would be approximately 0.5 for the association between CSF IL-6 concentration and the z score, which would require 25 patients to obtain a power of 80%. Because of the risk of incomplete data sets or dropouts, we aimed to enroll >30 patients.

The effects of surgery on changes in CSF and serum biomarkers from preoperative levels were tested by a repeated measurements analysis of variance, and paired t tests were used to assess significant changes from preoperative levels at each postoperative time point. We performed a principal component analysis (PCA) on the preoperative CSF and blood biomarker measurements. Fifty-two of 92 markers, for which less than 25% of the subjects' preoperative measurements were at the lower detection limit, were included in the PCA.

In brief, a principal component (PC) can be seen as a weighted average of the Z-transformed measurements of all 52 markers:

$$\mathrm{PC} = \sum_{1}^{52} w_i \times Z_i, \text{where } Z_i = X_i - \frac{\overline{X}}{SD} \text{ and } w_i \text{ is the weights obtained from the PCA}$$

Because the Z_i's have means that equal zero, the PC also has mean of zero and a standard deviation (SD) depending on the weights (w_i). The weights reflect the PC's correlation to each of the individual markers, where large positive and negative weight implies positive and negative correlation, respectively.

Analysis of the Postoperative Outcome

The 3 first PCs from the preoperative PCA were a priori selected as primary outcome variables. Postoperative outcome variables were then created by applying these weights on the Z-transformed markers at each postoperative time point for each variable. These longitudinal outcome variables were analyzed by a random slope and random intercept repeated measurement model with an unstructured covariance matrix. This model included the preoperative value of the PC as a covariate, and the group–time interaction was assessed to test a difference in the time course for patients with or without long-term cognitive decline. The analyses of individual CSF and blood biomarkers were carried out with a similar model, with the exception that time was considered as a categorical variable. Complementary to the group–time interaction test, a Fisher exact test was applied to compare proportions with positive slopes in the PC2 analysis with respect to cognitive outcome.

As a sensitivity analysis, we randomly divided the patients into 12 subsets and repeatedly performed PCAs by leaving out 1 subset at a time. The PC2 from each analysis was then applied to the postoperative measurement for all patients, and the time course for the 2 patients left out from each PCA was compared to the time course obtained from the PC2 based on all patients. Furthermore, the group–time interaction tests from each of the 12 analyses were performed (Supplementary Table 2). Differences between groups at the various time points, postoperatively, were tested by t test, allowing for unequal variances between groups in cases when applicable. Bonferroni–Holm correction was applied when

testing differences in preoperative levels for individual CSF and blood biomarkers with respect to long-term cognitive outcome and for tests of group–time interactions for individual blood biomarkers. Postoperative longitudinal changes of the biomarkers of astroglial injury and the CSF/serum albumin ratio were analyzed statistically with a linear mixed model, using an autoregressive covariance matrix and random intercept, comparing changes from baseline over time after surgery and the group–time interaction. A 2-way analysis of variance for repeated measurements was used for analysis of quality of sleep and the severity of postoperative pain (VAS). All data on pre- and intraoperative patient characteristics are presented as median and interquartile range (Q1, Q3). Data on biomarkers are presented as means and standard error of the mean (SEM) and group differences tested by Mann–Whitney *U* test and Fisher exact test. A $p < 0.05$ was considered significant. The analyses were performed by PROC PRINCOMP, PROC MIXED, and PROC TTEST with software SAS version 9.4 (SAS Institute, Cary, NC).

Results

The clinical trial profile is shown in Figure 1. Out of 156 patients assessed for eligibility, 34 patients entered the study. Of these, 7 patients were excluded for reasons described in Figure 1, providing 27 patients for final analysis.

Neurocognitive Outcomes and Perioperative Patient Characteristics

At 3 months postsurgery, 6 of 27 (22%) showed a composite *z* score of ≥1.0 (poor neurocognitive outcome group, n = 6), among which 2 had a composite *z* score of >1.96.

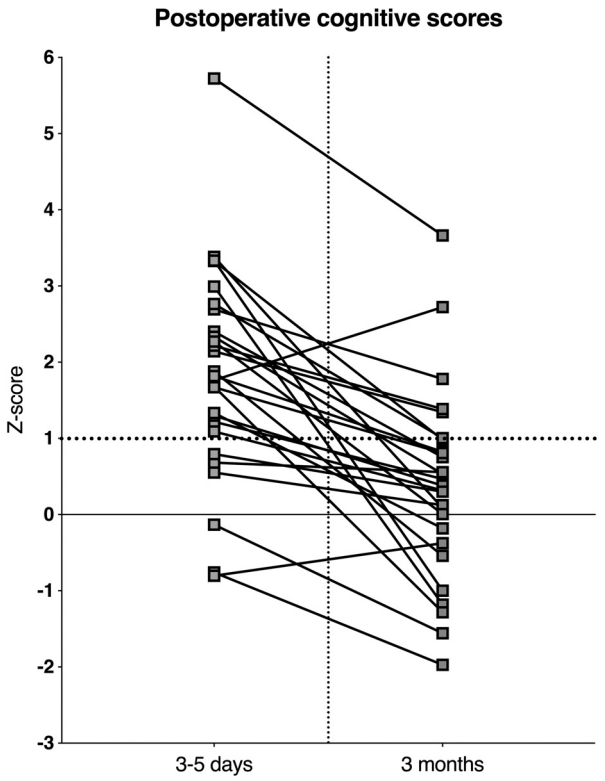

FIGURE 2: Individual (n = 27) neurocognitive outcome trajectories at hospital discharge (postoperative day 3–5) and at 3 months after major surgery. At 3 months postsurgery, 6 of 27 patients (22%) showed a composite *z* score of ≥1.0 (long-term cognitive decline).

The remaining 21 patients (78%) had a composite *z* score of <1.0 (good neurocognitive outcome group, n = 21).

At hospital discharge (3–5 days postsurgery), 21 patients (78%) showed a composite *z* score of ≥1.0, among which

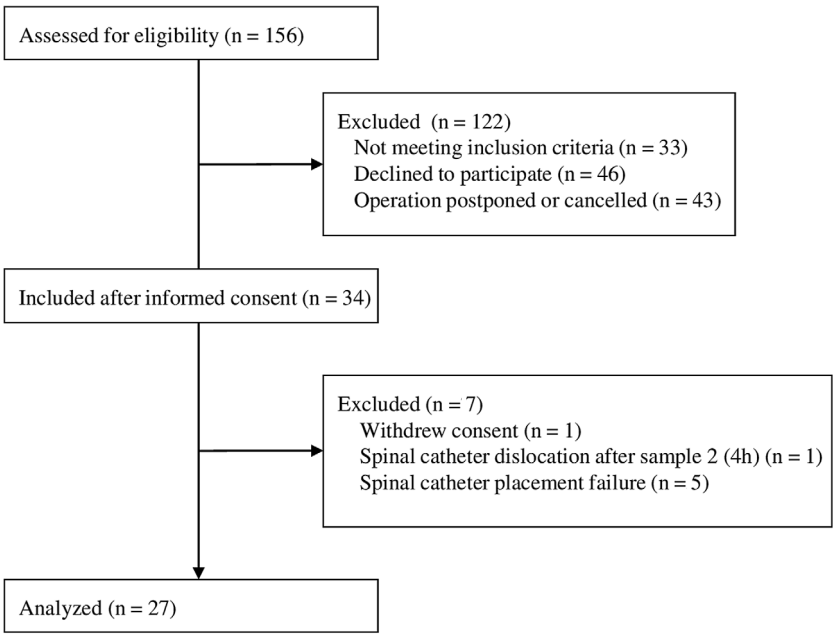

FIGURE 1: CONSORT (Consolidated Standards of Reporting Trials) diagram showing patient inclusion.

13 patients (48%) had a composite *z* score of >1.96. Individual neurocognitive trajectories are presented in Figure 2.

Patient demographics and comorbidities are presented in Table 1. There were no differences between patients in the good versus poor neurocognitive outcome groups with respect to demographic variables, comorbidity burden, American Society of Anesthesiologists physical status classification, preoperative hemoglobin, serum creatinine, or ongoing medication. Perioperative patient characteristics are described in Table 2.

TABLE 1. Demographic and Comorbidity Characteristics by Neurocognitive Outcome at 3 Months

Characteristics	Good Neurocognitive Outcome, n = 21	Poor Neurocognitive Outcome, n = 6	*p*
Age, yr	71 (65–76)	68 (65–71)	0.32
Sex, male, n (%)	8 (38)	1 (17)	0.63
Weight, kg	80 (73.5–89)	79 (75–88.5)	0.93
Height, cm	171 (167–179)	166 (163–175)	0.24
Body mass index, kg/m^2	26.7 (24.6–29.4)	27.9 (25.7–31.5)	0.44
Comorbidity			
Hypertension, n	11	4	0.66
Diabetes mellitus, type 1, n	2	0	1
Diabetes mellitus, type 2, n	0	1	0.22
History of myocardial infarction, n	1	0	1
Active smoker, n	0	0	NA
ASA classification			0.197
I, n	7	0	—
II, n	13	6	—
III, n	1	0	—
IV, n	0	0	—
Blood hemoglobin, g/l	138 (128–147)	143 (134–157)	0.41
Serum creatinine, μmol/l	71 (62–89)	68 (47–96)	0.63
Preoperative WBC count	6.3 (5.4–7.9)	7.7 (6.5–8.7)	0.22
Ongoing medication			
Beta-adrenergic blocker, n	3	1	1
Calcium channel blocker, n	4	3	0.29
Angiotensin converting enzyme inhibitor, n	7	0	0.15
Angiotensin II receptor antagonist, n	4	2	0.59
Insulin + oral antidiabetic, n	2	0	1
Oral antidiabetic, n	0	1	0.22
Acetaminophen, n	6	2	1
Opioids, n	0	0	NA

Values are median (Q1–Q3). Group differences tested by Mann–Whitney *U* test and Fisher exact test.
ASA = American Society of Anesthesiologists; NA = not applicable; WBC = white blood cell.

TABLE 2. Perioperative Patient Characteristics by Neurocognitive Outcome at 3 Months

Characteristics	Good Neurocognitive Outcome, n = 21	Poor Neurocognitive Outcome, n = 6	*p*
Intraoperative			
Propofol, mg	170 (80–291)	168 (75–288)	1.0
Fentanyl, n	4	0	0.55
Alfentanil, n	2	0	1.0
Ketorolac, local infiltration, n	5	1	1.0
Vasopressor, n	12	5	0.36
Intravenous fluids, ml	1,200 (950–1,350)	925 (750–1,560)	0.44
Blood transfusion, n	0	1	0.22
Duration of procedure, min	89 (72–102)	86 (72–101)	0.93
Procedure, n			
Hip replacement	14	5	0.63
Knee replacement	7	1	0.63
Bleeding, ml	300 (150–450)	225 (150–550)	1.0
Postoperative, 24 h			
PACU length of stay, min	165 (98–225)	171 (128–579)	0.47
Intravenous fluids, ml	1,000 (750–1,850)	1,575 (260–2,500)	0.48
Medication, n			
Gabapentin	6	2	1
Oral opioid	20	6	1
Intravenous opioid	13	3	0.66
Pain assessment, VAS score			0.09
4 h	1 (0–4)	0 (0–4)	—
8 h	6 (4–8)	6 (3–7)	—
24 h	6 (5–8)	6 (5–8)	—
32 h	5 (3–7)	8 (6–8)	—
48 h	4 (2–5)	6 (6–8)	—
Sleep assessment, VAS score			0.92
24 h	5 (3–7)	5 (3–9)	
48 h	3 (2–6)	5 (3–6)	—
Postspinal puncture headache, n	0	1	0.22
Blood patch, n	0	1	0.22

Values are median (Q1–Q3). Group differences tested by Mann–Whitney *U* test and Fisher exact test. A 2-way analysis of variance for repeated measurements was used to assess differences between groups with respect to quality of sleep and the severity of postoperative pain (VAS).
PACU = postanesthesia care unit; VAS = visual analog scale.

There were no differences between the 2 neurocognitive outcome groups with respect to administration of sedatives, vasoactive drugs, fluid management, intraoperative bleeding, duration and type of procedure, or length of stay in the post-anesthesia care unit. The VAS score for assessment of pain and sleep quality did not differ between the 2 groups, and no patient developed postoperative delirium.

The use of ketorolac was evenly distributed between the groups with good (24%) and poor (17%) neurocognitive outcome (see Table 2).

One patient with long-term cognitive decline developed postspinal headache and was successfully treated with an epidural blood patch.

CSF and Serum Biomarkers of Inflammation

Twenty-eight CSF and 14 serum biomarkers had a high fraction of measurements lower than detection limit and were not further analyzed. Supplementary Table 3 displays data on 64 CSF and 76 serum biomarkers irrespective of neurocognitive outcomes. Forty-seven CSF (73%) and 62 (79%) serum biomarkers showed overall significant 48-hour postsurgery changes from baseline (ie, a significant time effect), and many of the remaining markers showed a significant change from baseline on at least 1 postoperative time point (see Supplementary Table 3).

CSF and Serum Biomarker Response to Surgery in Patients with Good versus Poor Postoperative Neurocognitive Outcome

Out of 27 patients enrolled, 3 patients lacked 1 or more postoperative CSF samples, thus analysis of inflammatory biomarkers was performed in 24 patients. At first, preoperative CSF and serum biomarker levels were analyzed to explore the association between preoperative levels of inflammatory molecules and neurocognitive outcomes at 3 to 5 days and at 3 months postsurgery. After correction for multiple comparisons, none of the detectable inflammatory biomarkers measured preoperatively in either CSF or blood showed an

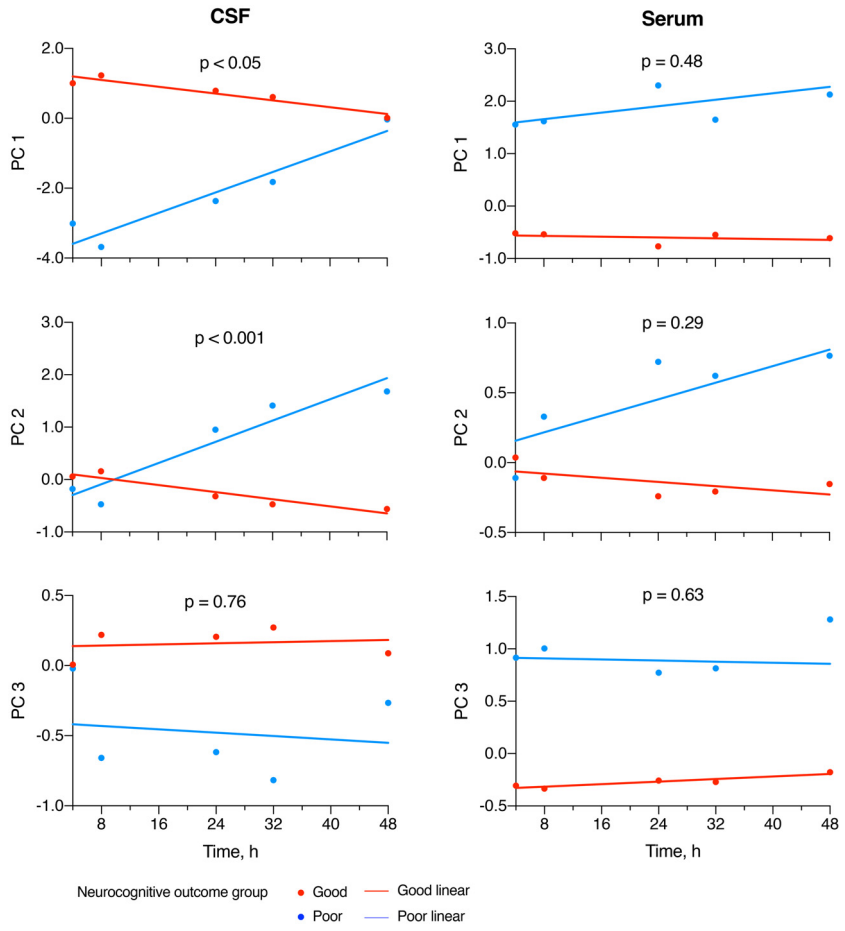

FIGURE 3: Preoperative principal components (PCs) 1–3 based on all 52 inflammatory biomarkers as construct postoperative outcome variables assessing the group–time interaction in cerebrospinal fluid (CSF) and serum (n = 24). The 2 first PC (PC1 and PC2) construct outcome variables on CSF samples were significantly associated with long-term cognitive decline at 3 months (poor). CSF biomarkers of inflammation increased the first 48 hours after surgery in the poor neurocognitive outcome group, and the opposite was seen in the good neurocognitive outcome group. In the corresponding analyses based on serum, none of the 3 PC-construct variables show a significant association to poor neurocognitive outcome.

association with neurocognitive outcomes at 3 to 5 days or 3 months (Bonferroni–Holm correction).

To overcome a potential mass-significance problem when assessing the relationship between temporal changes of biomarkers in CSF and serum to neurocognitive outcomes, as the next step, a PCA was performed on the preoperative measurements, irrespective of neurocognitive outcomes. The PCA was performed on the subset of 52 CSF markers for which at least 75% of the subjects had a preoperative value above the lower detection limit. Based on the loadings for the first 3 PCs, explaining 63% of the preoperative biomarker variation (that is, 49%, 8%, and 6% for PC1–PC3, respectively), 3 time-dependent outcome variables were created (PC1–PC3). For the 2 first of these construct outcome variables (PC1 and PC2), the overall time course patterns of the biomarkers were significantly different with respect to neurocognitive outcomes after 3 months (Fig 3; group–time interaction: p = 0.02, p = 0.00005, and p = 0.76 for PC1–PC3, respectively). All 6 patients with long-term cognitive decline showed an increase over time for the PC2 constructed outcome variable, but such an increase was detected in only 3 out of 18 patients without long-term cognitive decline (100% vs 17%, p = 0.006; Fig 4). For the same PCA construct variables, we could not detect any association with neurocognitive outcomes at days 3 to 5 postsurgery.

In a corresponding PCA on the same biomarkers in serum samples, the 3 first components explained 39% of the preoperative biomarker variability (18%, 11%, and 10% for PC1–PC3, respectively). None of these 3 PC construct variables in serum was significantly associated with neurocognitive outcome (see Fig 3; group–time interaction: p = 0.48, p = 0.29, and p = 0.63 for PC1–PC3, respectively).

In the sensitivity analyses, the time courses obtained when the subsets of patients were left out from the PCAs were virtually unchanged compared to the results based on all patients, and the group–time interaction tests based on the 12 data sets were all statistically significant (see Supplementary Table 2).

The relative contribution of individual biomarkers to the separation of the 2 CSF inflammatory trajectories was analyzed (Supplementary Table 4). When exploring the time dependence of individual cytokines and chemokines, IL12B, IL18, IL6, IL8, TGFB1, and TNFRSF9 showed a significant time–group interaction, and 10 out of 16 chemokines displayed a significant time–group interaction. Two out of 6 neurotrophic/growth factors (HGF, NGF), CDCP1, EIF4EBP1, MMP1, and 1 leukocyte cell surface biomarker (CD244) showed a significant time–group interaction. Only 3 of the individual serum biomarkers (LIF, TNFRSF11B,

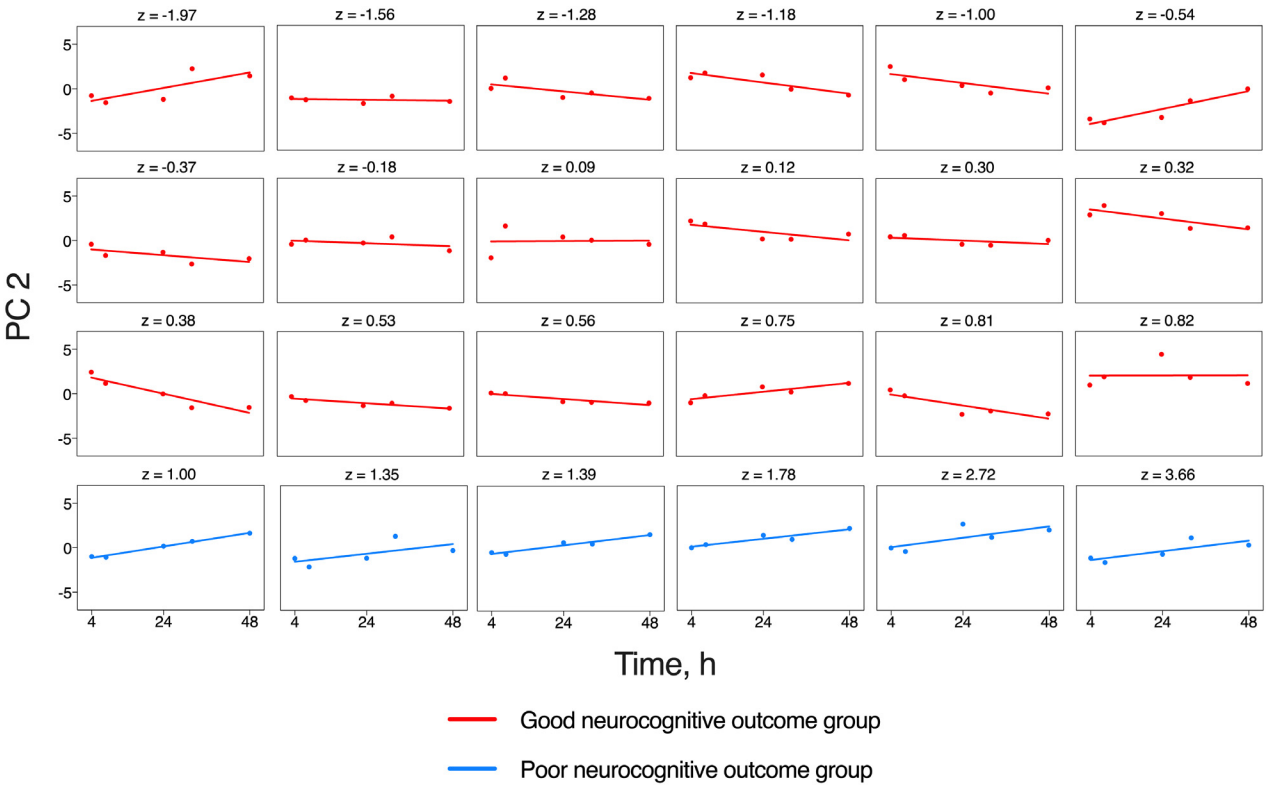

FIGURE 4: Individual regressions for the second principal component (PC2) based on all 52 cerebrospinal fluid (CSF) markers (n = 24). Individual regressions for PC2 construct outcome variables revealed that all patients with long-term cognitive decline (blue, n = 6) showed an increased CSF inflammatory trajectory over time, but only 3 of 18 patients without long-term cognitive decline (red, n = 18) showed this inflammatory CSF trajectory (p = 0.006; Z indicates individual z score).

and CCL23) showed nominally significant group–time interaction, and only LIF was significant after Bonferroni–Holm correction ($p < 0.05$).

Initial and Late Temporal CSF Biomarker Responses to Surgery in Patients with and without Long-Term Cognitive Decline

In a detailed analysis, the biomarker time-course patterns for PC1 showed a differential trajectory initially after surgery with respect to the development of long-term cognitive decline. The majority of the PC1 loadings had approximately equal size, and, as shown in Supplementary Table 4, for the vast majority of the biomarkers the response to surgery in the early phase (ie, the difference between 4 and 8 hours and baseline) was lower in the group with long-term cognitive decline (see Supplementary Table 4). Even though this pattern was consistent, only a few individual markers (12/52) showed a nominally significant difference between the groups (indicated by asterisks on the sign).

Time-course patterns for the highly significant PC2 showed the reverse pattern, with a pronounced difference in the end of the observation period (32 and 48 hours postsurgery). This was supported by the significantly higher late response in the group with long-term cognitive decline for markers with high PC2 loadings, for example, IL6, IL8, CCL3, CCL8, and CXCL6, as seen in Supplementary Table 4 and in Figure 5. In addition to biomarkers with high PC2 loadings, we detected additional biomarkers with significant separation at 48 hours between patients in the good versus poor neurocognitive outcome groups (IL12B, IL18, TGFB1, CCL3, CCL8, CXCL5, CXCL6, and HGF; see Supplementary Table 4).

Pathway Analysis

CSF biomarkers either with the highest loadings in PC2 or with significant separation between the good and poor neurocognitive outcome groups at 48 hours were analyzed for the enrichment in biological signaling pathways with online tools using Kyoto Encyclopedia of Genes and Genomes (KEGG) and WikiPathways databases, as well as Ingenuity Pathway Analysis software (Qiagen, Hilden, Germany). All database analyses produced similar results

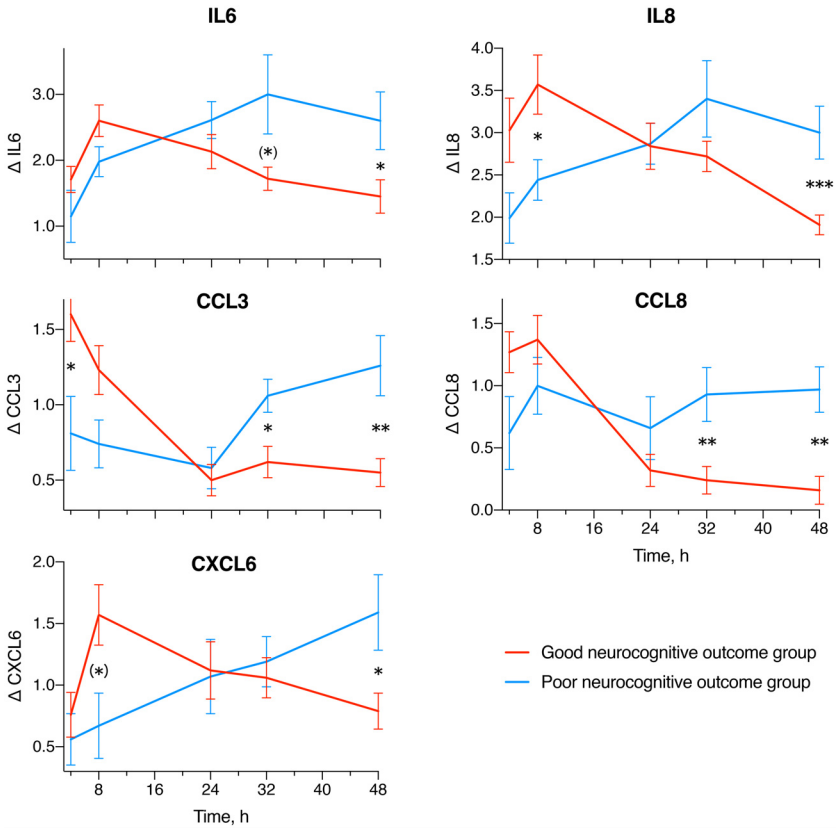

FIGURE 5: Postoperative changes in cerebrospinal fluid (CSF) levels of 2 cytokines (IL6 and IL8) and 3 chemokines (CCL3, CCL8, and CXCL6) with respect to neurocognitive outcome (n = 24). In patients with good neurocognitive outcome at 3 months, increased CSF levels of the biomarkers were seen 4 to 8 hours after surgery followed by a resolution at 32 and 48 hours. In contrast, in patients with long-term cognitive decline, CSF biomarker levels increased over time and were higher at the later phase (32 and 48 hours) postsurgery compared to patients without long-term cognitive decline (good). *$p < 0.05$, **$p < 0.01$, $^{(*)}p < 0.1$. Data are presented as mean ± standard error of the mean.

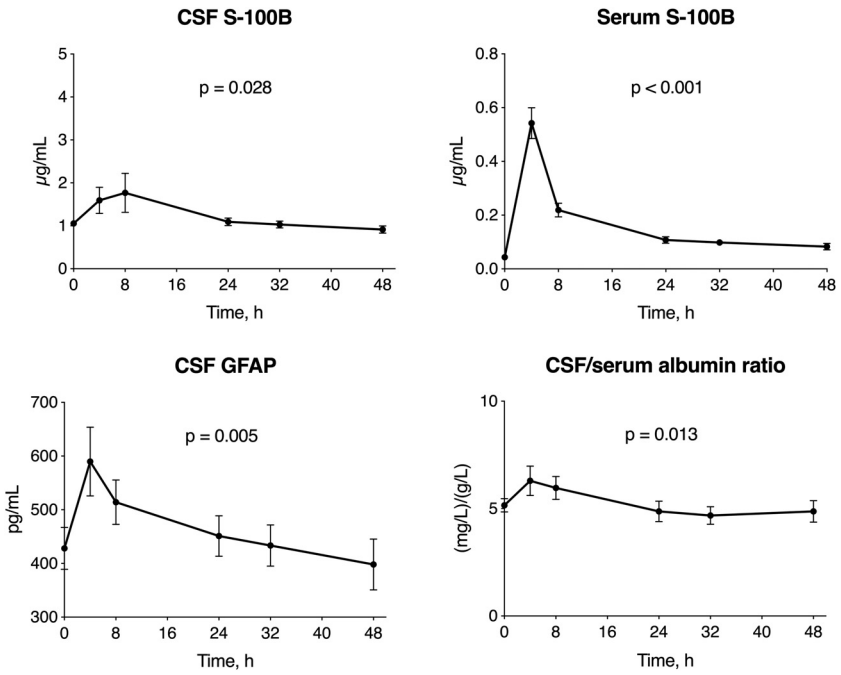

FIGURE 6: Temporal changes in cerebrospinal fluid (CSF) and serum markers of astroglial injury and blood–brain barrier function after major surgery (n = 27). The astroglial biomarker S-100B showed a transient increase at 4 and 8 hours postoperatively both in serum and CSF. At 24 hours postoperatively, both CSF and serum S100B were normalized. There was also a transient increase in CSF levels of glial fibrillary acidic protein (GFAP) and increased blood–brain barrier permeability (CSF/serum albumin ratio) at 4 and 8 hours postsurgery. There was no association between these changes in astroglial injury biomarkers or the CSF/serum albumin ratio and neurocognitive outcomes at 3 months postsurgery. Data are presented as mean ± standard error of the mean.

and showed particularly strong associations of chemokine signaling, cytokine–cytokine receptor interaction, and toll-like receptor signaling pathways with long-term cognitive decline. This is consistent with the overrepresentation of the chemokines in the group of high PC2 loadings biomarkers.

CSF and Serum Markers of Astroglial Injury and BBB Function

Serum S-100B showed a transient increase at 4 and 8 hours postoperatively ($p < 0.001$), and a similar pattern was noted in CSF ($p = 0.028$). There was also a transient increase in CSF levels of GFAP ($p = 0.005$) and BBB permeability (expressed as CSF/serum albumin ratio) at 4 and 8 hours postsurgery ($p = 0.013$). None of these changes in CSF or serum were associated with neurocognitive outcomes at 3 to 5 days or 3 months postsurgery.

Discussion

The major finding of this explorative study was that the time course of levels of inflammatory biomarkers in CSF differed between patients with cognitive decline at 3 months postsurgery and patients without cognitive decline. In the latter group, an initial early (4–8 hours) increase in CSF biomarkers of inflammation was followed by a gradual decline. In contrast, a divergent temporal pattern was uncovered in patients developing long-term cognitive decline; in these patients, an initial relative decline in CSF markers of inflammation was followed by continuous increase of inflammatory biomarkers throughout the 48-hour study period. In contrast, corresponding analyses of serum biomarkers showed poor association with long-term cognitive outcomes.

To our knowledge, this is the first study that describes an acute CSF inflammatory biomarker pattern in response to major surgery that closely associates with long-term cognitive outcomes. Our data suggest that preoperative levels of inflammatory molecules in either CSF or blood have little impact on neurocognitive outcomes, as there was no association between levels of cytokines and chemokines prior to surgery and later changes in individual cognitive performance. This may preclude preoperative assessment of baseline biomarker patterns as preventive strategy.

The separation between inflammatory trajectories rapidly becomes apparent once surgery has triggered the innate immune system and reached the CNS. Notably, the data display a very robust difference in the biomarker response pattern in CSF in relation to long-term neurocognitive outcome. Our findings also suggest that long-term brain outcomes are dependent on mechanisms related to inflammatory resolution, which seems critical for the recovery of cognitive capacity.

A small number of CSF cytokines, inflammatory cell-mediated biomarkers, and a broader array of chemokines contributed significantly to the differential response pattern in CSF. Common denominators for the biomarkers showing high PC loadings and significant differences between the 2 patient groups at the early and/or late time points are their chemo-attractive and differentiation-inducing features on innate immune cells, that is, myeloid cells and polymorphonuclear cells. In patients with long-term cognitive decline, higher CSF levels of IL-6, IL-8, CCL3, CCL8, and CXCL6 at 48 hours after surgery (see Fig 5), a time point when the BBB permeability had returned to presurgery status (Fig 6), suggest that immune cells within the CNS produce these mediators. Furthermore, the significant contribution from CX3CL1 (fractalkine), a neuron-enriched biomarker, during the first 8 hours suggests early neuronal involvement (see Supplementary Table 4). IL-6 has, in addition to its multiple inflammatory functions, also been described as causing reduced hippocampal neurogenesis in rodent models. Inhibitors of IL-6 are clinically available, both as an antibody binding to sIL-6R and thereby inhibiting IL-6 signaling and as a small molecule inhibiting JAK/STAT signaling, resulting in less IL-6 production. It is tempting to speculate that such anti-inflammatory treatment immediately after surgery could affect the long-term cognitive outcome.

Major surgery is accompanied by increased CSF levels of astroglial cell injury markers, for example, GFAP and S-100B,[8] and short-lasting alterations of BBB permeability, as assessed by an increase in the CSF/serum albumin ratio.[8,9] Those findings were confirmed in the present study, suggesting a surgery-induced astroglial injury and increased BBB permeability (see Fig 6). However, neither the CSF release of the astroglial injury markers nor the increased BBB permeability was associated with long-term cognitive decline; therefore, we propose that this condition is not primarily ascribed to a more pronounced disruption of the BBB integrity.

Although Hirsch et al[7] found substantial increases in CSF and blood concentrations of proinflammatory cytokines during the first 18 hours postsurgery, they were unable to detect an association with changes in cognitive outcomes at 1 to 3 days postsurgery. In line with these observations, we confirmed that there was no association between either CSF or serum biomarker patterns and early neurocognitive outcomes at 3 to 5 days postsurgery, indicating that other factors that adversely affect cognitive performance in the immediate (1–3 days) postoperative period such as postoperative pain, sleep disturbances, and drug effects[17] are more important. In a recent PET study of the human brain after surgery, an early and profound downregulation of the brain immune activity was demonstrated followed by a recovery at 3 months, and in some patients an increase above baseline was observed, suggesting a late upregulation of the immune activity.[14] The magnitude of this upregulation correlated to the decline in some aspects of the cognitive function. The present results are in line with these findings; patients having a poor cognitive performance at 3 months display steadily increased levels of CSF biomarkers of inflammation in contrast to those patients with recovered cognitive function.

Interestingly, although a close association between CSF biomarker pattern and long-term neurocognitive outcomes was found, there was no support for a difference in the trajectories of the systemic inflammatory response between patients with good versus poor neurocognitive outcome. This finding may be due to a limited power to detect a differential trajectory within the systemic circulation, or it might indicate that other systemic conditions are important determinants of biomarker concentrations in blood after a surgical trauma. These results are in line with a recent meta-analysis on the association between peripheral inflammatory markers and postoperative cognitive dysfunction showing that plasma IL6, but no other peripheral cytokine, was associated with postoperative cognitive dysfunction.[18] Moreover, our findings confirm recent findings by Forsberg et al[14] of a lack of association between changes in plasma cytokines postsurgery and cognitive performance. Hence, it may be argued that the use of plasma measurements of acute inflammatory markers in the early postoperative period (hours) may have a limited utility for prediction of late postoperative neurocognitive decline. However, we cannot exclude the possibility that serial blood measurements of inflammatory biomarkers at later time points (days to weeks postsurgery) would reflect long-term neurocognitive outcomes.

PCA was applied to identify a set of new independent variables explaining most of the preoperative biomarker variability, as analyses of individual biomarkers would be hampered by mass significance due to the high dimension of explanatory variables. We chose to analyze the 3 first PCs, explaining 63% of the total preoperative biomarker variation in CSF. The obtained PCs are linear combinations of the standardized biomarker measurements, where the coefficients (often called loadings) for each biomarker are obtained from the PCA so that the individual components are statistically independent of each other. The PCs may represent latent processes causing correlation between individual biomarkers in absence of the surgical trauma. By applying the identified PCs to the postoperative measurements in relation to cognitive outcome, we hypothesized that the latent structures

identified would be differentially affected by the surgical trauma. In this study, PC1 had about equally sized loadings for the vast majority of the 52 biomarkers included and thus can be interpreted as the average standardized expression of all markers. PC2 was most strongly associated with cognitive outcome and had high positive or negative loadings for a limited subset of markers; postoperative separation of these markers with respect to cognitive outcome will primarily contribute to the observed separation of PC2. The significant associations to cognitive outcome found with the PCA approach was considered as a gatekeeping test, where we identified 6 primary outcome variables (three PCs in CSF and blood, respectively). Because the PCA approach yielded a significant association with postoperative biomarker expression in CSF, no further adjustment was made for multiple testing when analyzing individual CSF biomarkers. The interpretation of these results should therefore take into consideration the risk of type 1 and type 2 errors.

A major limitation of the present explorative study is the small sample size, with only 6 patients in the poor neurocognitive outcome group. Therefore, the findings of this study should be considered preliminary and requiring validation in a larger surgical patient cohort. Despite these caveats, there were consistent and highly significant qualitative and quantitative differences between the good and poor neurocognitive outcome groups with respect to the brain immune response to a standardized elective surgical trauma.

The definition of postoperative decline varies across studies. We decided to classify patients according to a composite z score higher than 1, which allowed us to also include patients with minor deterioration and increased the number of cases with the outcome of interest.

One could argue that the use of bupivacaine and sufentanil for spinal anesthesia and the indwelling spinal catheter may have caused an inflammatory reaction in the CSF. Although bupivacaine has been shown to exert anti-inflammatory effects in vitro on inflammation-activated astrocytes[19] and lipopolysaccharide-activated macrophages,[20] the effects of intrathecal sufentanil and IV sedation with propofol on CSF inflammatory biomarkers are unknown. The anesthetic regimen was uniform, and it is therefore unlikely that the different brain immune responses between the good and poor neurocognitive outcome groups could be explained by interindividual differences in the anesthetic management. Furthermore, there were no differences between the 2 groups with respect to pre-, intra-, and postoperative characteristics, which all could have had an influence on postoperative cognitive function. Finally, it should be acknowledged that increased blood concentrations of S100B may indicate potential release from extracerebral sources.

In conclusion, in this explorative study, we have shown that the trajectory of CNS inflammatory response after surgery differs quantitatively and qualitatively in patients with postoperative long-term cognitive decline versus those patients not developing long-term cognitive decline, and no such association to postoperative cognitive decline was shown for serum biomarkers of inflammation. These findings require validation in a larger surgical patient cohort.

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Author Contributions

M.D., A.W., F.G., K.B., S.M., B.N., J.O., M.J.F., L.S.R., H.Z., S-E.R, and L.I.E. contributed to the study concept and design. M.D., A.W., F.G., K.B., S.M., B.N., J.O., M.J.F., A.G., A.S., L.S.R., H.Z., S-E.R, and L.I.E. were involved in data acquisition or analysis. M.D., A.W., F.G., K.B., S.M., B.N., J.O., M.J.F., L.S.R., H.E.H., H.Z., S-E.R, and L.I.E. took part in drafting the manuscript and figures.

Potential Conflicts of Interest

Nothing to report.

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